

## Reverse Complement DNA Sequence 09•YD - 19.Seq(1,263)

...

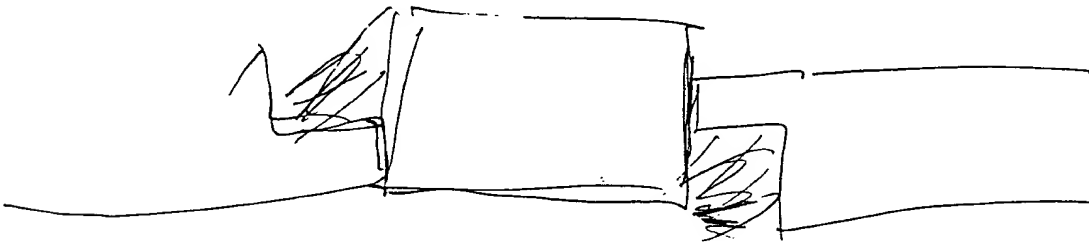
10 20 30 40 50 60

TTT TNG TTTT TTACCTCGGG TINGAAATCG ATCGGGATAA AACTAACAAA ATCGGTTATA 60  
CGATAACGGT CCGTACGGGA TTTTCCCATC CTACTTTTCAT CCCGGGCTAC AAGGCTTCCC 120  
AAGCTCACTC GGGAGCAACA GGATCTATTG TGGTGGAGTC GGGTCGGGTC AGGTTATGAT 180  
CGACCCCGGTT ATTCTCCAT GGGTTTGTGTT GAGACTTCCT TCC 223

3' - cDNA / 5' D3

10 20 30 40 50 60

TTTTCGTTTT TTACCTCGGG TTCNAAATCG ATCGGGATAA AACTAACANA ATCGGTTATA 60  
CGATAACGGT CGGTACGGGA TTTTCCCATC CTACTTTTCAT CCCGGGCTAC AAGGCTTCCC 120  
AAGCTCACTC GGGAGCAACA GGATCTATTG TGGTGGAGTC GGGTCGGGTC AGGTATATGAT 180  
CGACCCGGTT ATTTCTCCAT GGGGTTTTGT TGAGACCTCC TCCACTACTC ATGAGCTCTC 240  
TTCANT 246



10 20 30 40 50 60  
 GTAGCATCGA TCTCTAACAA CGCTACCCGT TTACCCGTAC CGGTAGACCC GGGTGTGTG 60  
 CTACAGGGAT GA<sup>A</sup>AACGGTC GGTAACGGTC GGTA<sup>A</sup>AATAC CTCTACCGTT TTCATTTTCA 120  
 TATTTAACTT GCGGGACGGA AACN<sup>A</sup>AAACG GGATATACCG GTAACN<sup>A</sup>AAA CGAACGGGAT 180  
 AAATACGGTA ATCGAGTGnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnTT TTCGTTTTTT 240  
 ACCTCGGGTT CNAAATCGAT CGGATAAAA CTAACANAAT CGGTATATACG ATAACGGTTCG 300  
 310 320 330 340 350 360  
 TACGGGATT TTCCCATCCT AC<sup>T</sup>TTTCATCC ~~CGGCTACAA~~ GGCTTCCCAA GCTCACTCGG 360  
 GAGCAACAGG ATCTATTGIG GTGGAGTCGG GTCGGGTCAG GTTATGATCG ACCCGGTTAT 420  
 TTCTCCATGG GGTTTGTGTG AGACCTCCTC CACTACTCAT GAGCTCTCTT CANT 474

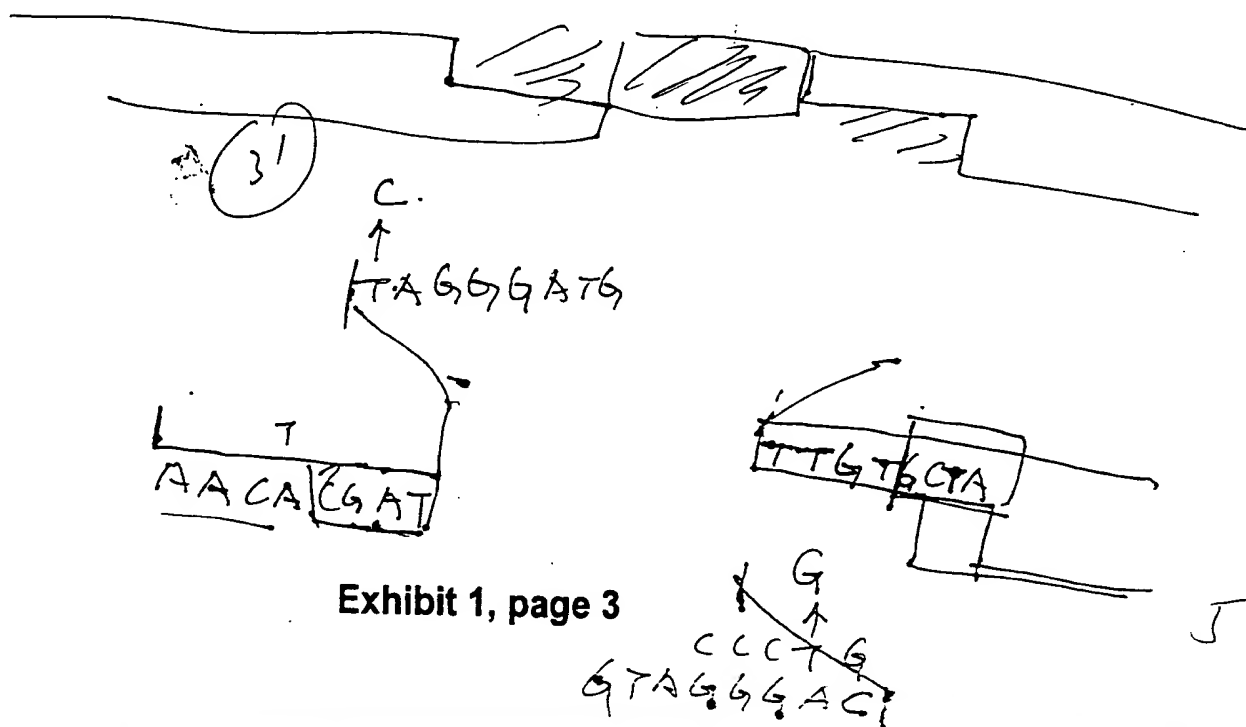


Exhibit 1, page 3

## Reverse Complement DNA Sequence 22•YWC - 282-33-2..Seq(1,699)

...

10 20 30 40 50 60  
 AAAGGAGAAA GAAAGAAGGA AAGGAAAANA GNAGGGGGGA AAGAAANGGN NANGAGNNAA 60  
 GAAGANAGGA AGTGAAGGGG AGGGGAAGNG GAGAAAGGGG GAANGNTTAA TTTTANGINA 120  
 GNGGINNNAA ATTCTGIGAG AAACCCNGGT GATTTTATGA GGGACACCNG TGTTATNGTC 180  
 AATAGANNAN GAGAGATNCG GACAGAGACA CTGAAGAGIN NINGINGGAA ACCAGGATCG 240  
 ^AGACANGTC AACAGAGACN GNGNAACCA ACGTTGAGAG GAATGGGINN AGCAGAGGTC 300  
 310 320 330 340 350 360  
 GANCGICAGA GAATNGNAGN AGAAAAGAAG CAANTCACCN CCNCCACAGT CGGAGACACG 360  
 TCATCAGTAN CNINGATATC TAACCACGTT ACCCGTTINAC CCGTACCGGT AGACCCGGGT 420  
 GTTGTGCTAC AGGGATGAAA ACCNTCTGGT ANCGGINGGT TATATACATT TAACCTTGTT 480  
 TNGINTTINA AAGINAACIN TGAGNGNCGT GAA 513

# Large scale production of Fluorescein Sequin

- use the original 1:50 solution of 2nd round T-PCR product. ~~80~~ 78 81 33 and redilut of 36/2:50

- 78 (<sup>82</sup>SGT ~~78~~): D55-3/AD3 12
- 36 (SGT 736): D53-3/AD3 12
- 81 (SGT 736): D55-3/AD3 24
- 33 (SGT 282): D53-3/AD3 24

Cocktail:

A: for 33 and 36: 37 sets

B: for 78 and 81: 37 sets

	D53-3/AD3	D55-3/AD3
x Buffer	74	74
gel2	59.2	59.2
trio	432.9	432.9
dNTP	6.0	6.0
Ds primer	14.8	14.8
AD primer	74.0	74.0
poly.	5.5	5.5
	6 6 6.4	6 6 6.4

33: ?	444.2 ul	81: ?	444.2 ul
WA: ?	24.6 ul	WA: ?	24.6 ul

Exhibit 2, page 1

36: ?	222.1 ul	78: ?	222.1 ul
-------	----------	-------	----------

Large scale production of 282/736 Flaming 3/5

Test running. 2% agarose gel. 282

100 µl eluted  
20 µl 2nd AP  
20 µl 3rd AP  
20 µl 4th AP  
20 µl 5th AP  
20 µl 6th AP  
20 µl 7th AP  
20 µl 8th AP  
20 µl 9th AP  
20 µl 10th AP  
20 µl 11th AP  
20 µl 12th AP  
20 µl 13th AP  
20 µl 14th AP  
20 µl 15th AP  
20 µl 16th AP  
20 µl 17th AP  
20 µl 18th AP  
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20 µl 70th AP  
20 µl 71st AP  
20 µl 72nd AP  
20 µl 73rd AP  
20 µl 74th AP  
20 µl 75th AP  
20 µl 76th AP  
20 µl 77th AP  
20 µl 78th AP  
20 µl 79th AP  
20 µl 80th AP  
20 µl 81st AP  
20 µl 82nd AP  
20 µl 83rd AP  
20 µl 84th AP  
20 µl 85th AP  
20 µl 86th AP  
20 µl 87th AP  
20 µl 88th AP  
20 µl 89th AP  
20 µl 90th AP  
20 µl 91st AP  
20 µl 92nd AP  
20 µl 93rd AP  
20 µl 94th AP  
20 µl 95th AP  
20 µl 96th AP  
20 µl 97th AP  
20 µl 98th AP  
20 µl 99th AP  
20 µl 100th AP

Good



- OK.

- purify the rest of 33. D53-3/AD3  
81: D53-3/AD3

recovered: - 18 µl x 21 = 378 µl

added 6X loading buf. 73.6 = 451.6 µl.

- run 1% gel.

- cut out the bands. 433: 300 mg x 3

481: 300 mg x 5

Exhibit 2, page 2

- store the gel block at 4°C

O/W?

could be  
contaminated by 10%  
213 thrown by mistake  
using the block for  
233

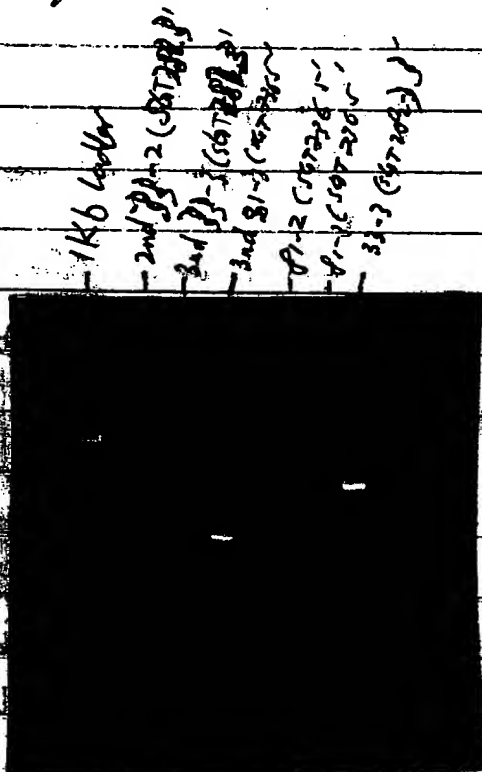
anyway, should not

Quick - gel column - purify the Fragment.

3 column / each sample. - multiple loading  
- twice wash.

- elute in  $50 \times 3 = 150$  ml. H<sub>2</sub>O

- run ~~10%~~  $\rightarrow$  10 ml in 2% agarose gel.



smaller: S6T736 5 $\frac{1}{2}$ /A0  
Larger S6T282 - 3 $\frac{1}{2}$ /A0

stored: Box # IL-1 - 20 $^{\circ}$ C

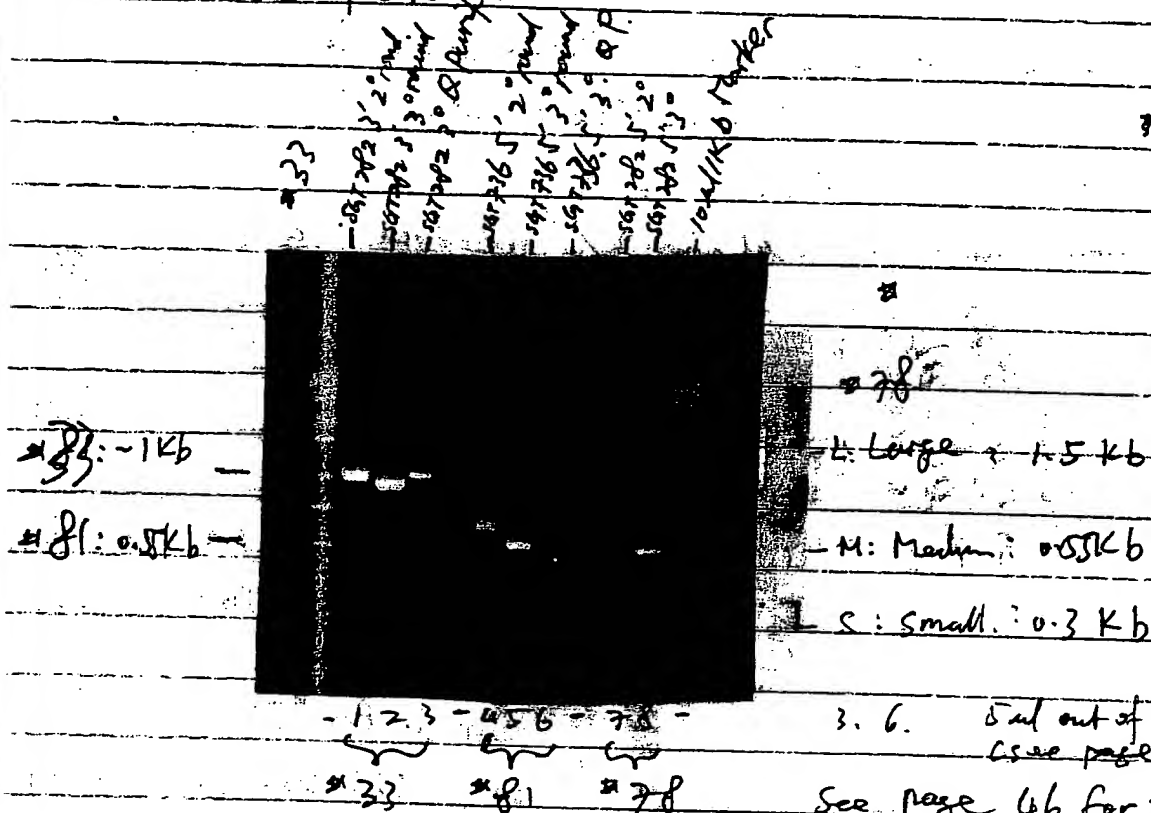
- S6T736 5 $\frac{1}{2}$ /AD3: #81-3 (Larger)

- S6T282 3 $\frac{1}{2}$ /AD3: #33-3 (Larger scale)

- run the second-round - large-scale - products - of  
#33, #81 and #78.  
Larger

see the result on page 54.

- the result of the 2nd. 3rd round of PCR of large scale PCR



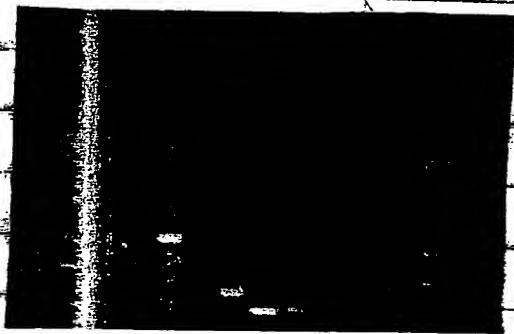
3. 6. 5' out of 50 and larger scale (see page 50-53)

See page 46 for the background.

### Notes:

- the Q-purified bands<sup>(3)</sup> run slower (larger) than its original PCR product (2). Why? Cut out the #1 ~ 3 bands purified by Q-f column to check it out (see the photo below for excising the bands)

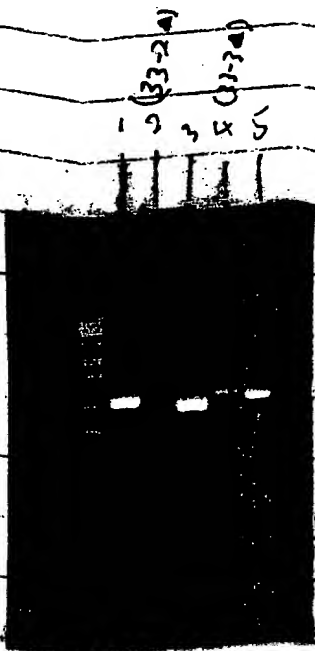
=. #78 positive is a biggest good news: SGT282: 5' Flanking  
 50. The Flanking sequences from both ends have been already out  
 3'-end Ds3/AD3: ~~cut~~ 3 bands: L 1.5 Kb, M 0.5 Kb, S 0.3 Kb  
 5'-end Ds5/AD3: single band: ~500pb



- Q-Q column purified the cut-bands of #33-2, #33-3, and #78-3.

- #78-3 (SGT282 5'/AD3) was passed to YC for cDNA screening

- run the #33-2, #33-3 compared with the PCR originals



SGT282 DS5/AD3

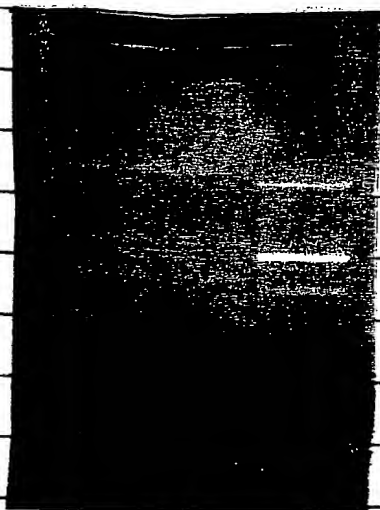
1. original 3' PCR product. 10 ul
2. Q-purified 2' PCR product. 10 ul out of 50 ul
3. original 3' PCR product 10 ul
4. Q-purified 3' PCR product 10 ul
5. 5 ul Q-purified 3' round Larger scale production.

Conclusion OK

stored: Box # FL-1 -20°C

SGT282 5'-FL. 2' round (2) : 33-2

SGT282 3'-FL. 3' round (4) : 33-3



P-Lab. meeting: on duty. Larkin

SGT 282

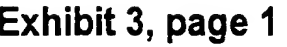
- run the purified the SGT282-5' Flakes
- cut out the 3 different sized bands
- stored at 4°C

Exhibit 2, page 5

Q-purification. also in ...

Enzymes: All 206 enzymes (No Filter)

Settings: Linear, Certain Sites Only Standard Genetic Code



Alu I  
Hpa II

Bin I  
Dpn II  
Mbo I  
Xho II

Dpn I  
Bca77I  
BsaWI  
BspMI  
Alw26 I  
Hpa II  
BsmA I

EarI  
Ksp632 I  
Hinf I

Ple I  
Mbo II

EcoR II  
Eco57 I  
Apy I  
Sbf I  
Bin I  
Dpn II  
Mbo I  
Dpn I  
Taq I  
Bbv II

EcoP I  
Bbs I  
Bpu10 I  
Bsc91 I  
Mbo II

Mnl I  
Mae II  
Psp1406I

I A P A R S G Q R H . R V V V G N Q D R R Q V C K N R R N Q  
 . L R R D P D R D T E E S W S E T R I E D R S A K T E E T N  
 N S S G E I R T E T L K S R G R K P G S K T G L Q K Q K K P  
 +-----+  
 E A G A L D P C L C Q L T T T P F W S R L C T Q L F L L F W  
 Y S R R S G S L S V S S D H D S V L I S S L D A F V S S V L  
 L E P S I R V S V S F L R P R F G P D F V P R C F C F F G V

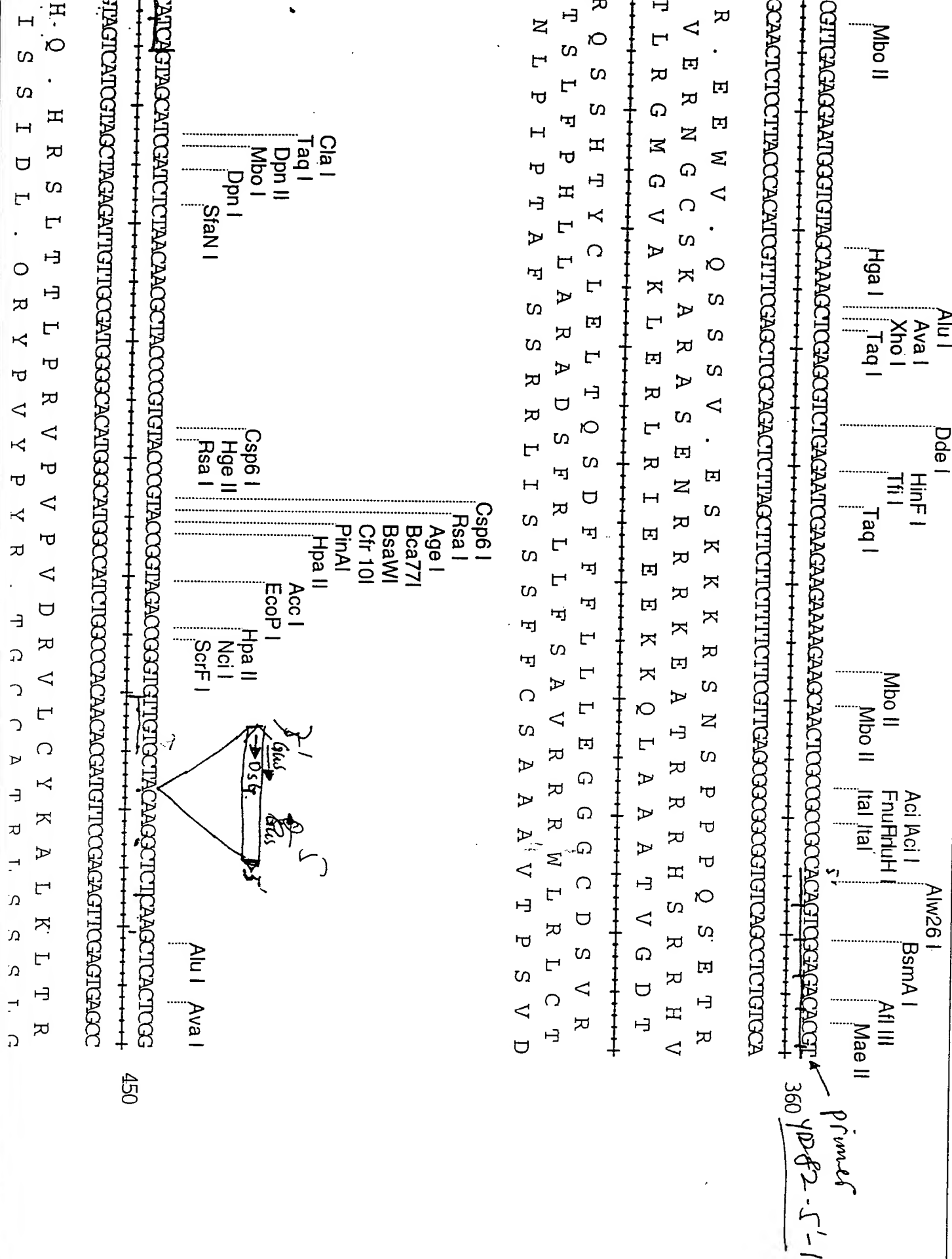
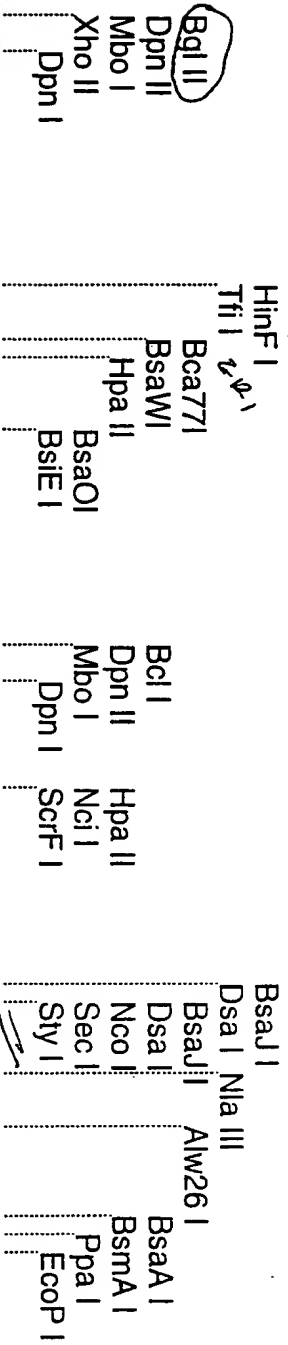


Exhibit 3, page 3

SSVASISNNATPCTRTGRRPGVVLQGSQAHS  
...YCRDRVVSGR TG TGTSRTNH. LARLSVR  
MLLM SR. CR. GTYGYRYVPHQA VLS ELES P  
DTADIELLA VGHVRVPLGP TTS CP E. A. EP



GAGCACAGATCTATGTGIGGATCGGTCGAGTATGATCAACCGGATTAATTCATCGGGTTGTGAGACTCTCTAC  
CTCGTGTCTAGATTAACCACTTACGCGACGCCAGTTCACTAGTTGGGCTAATTAAGAGTAACCCCAACAACCTCTGAGAGCTG  
EQQLDLWNPNPVGSSSMINPDYSPWGLLRPLY  
SNKIYCGGIRSGQV. STRIILHGV C. DLST  
GATRSIVVESGRVKYDQPGLF S MGFVETSL  
SCCSRNFHFGT PDLIILGS. EGH PKNLGR.  
LLLI. QPPI RDP. THDVRIIRWPTQQQSRE V  
AVL DITTS D PRTLYS. GPNNE MPNTSV ERS

540

40-28-3-1

primer 282-3'-1: GAAAGAAAGC, TCA, TGA, GTA. *Handwritten signature*

51

20.

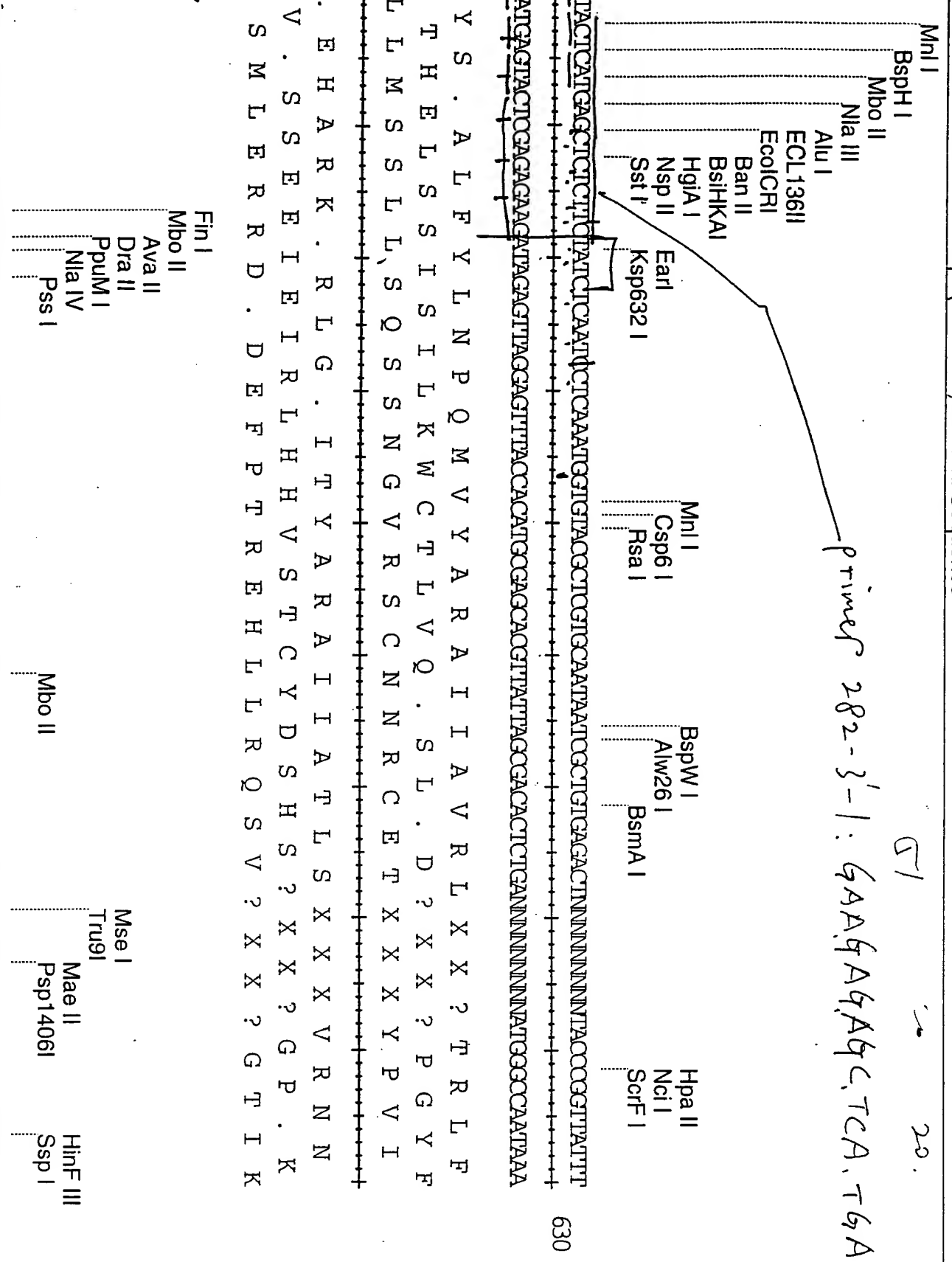


Exhibit 3, page 5

F Q W V W W D L L P K F R S F F Q S Q I F K C . T F F P I F  
F N G F G G T F F Q N S G V F F N L K S S N V K R F F Q Y S  
F S M G L V G P S S K I Q E F F S I S N L Q M L N V F S N I  
-----  
K . H T Q H S R R G F N L L K K . D . I K L H . V N K G I N  
K L P N P P V K K W F E P T K K L R L D E F T L R K K W Y E  
E I P K T P G E E L I . S N K E I E F R . I N F T K E L I R

Mae II  
Psp1406I

Mbo II  
Bin I  
Dpn II  
Mbo I  
Dpn I  
Fok I

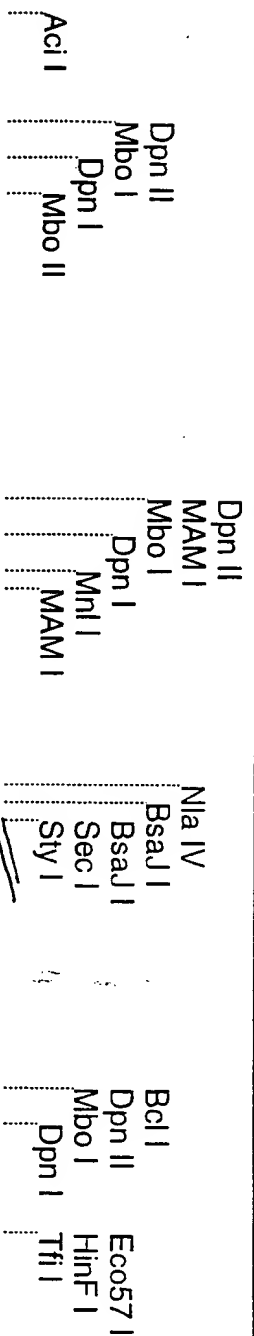
Taq I  
Bin I  
Dpn II  
Mbo I  
Dpn I  
BSL I  
BsiY I  
Asu II  
Taq I

Mbo II  
Mbo II  
Hinf I  
Tfi I

GCTGACACTTCTTCAGAGAAGACGTTTGATGGATCAGATATATGATGATTCGATCCAAAGGTTGGGATTTTGGAAAAACACAATCA  
-----  
CGAAGTGAAGAGTCTCTTTCGAACCTACCTAGCTTATTTACATCAAGCTAGTTGCCACCCCTAAAGCTTTTGTGTTACT

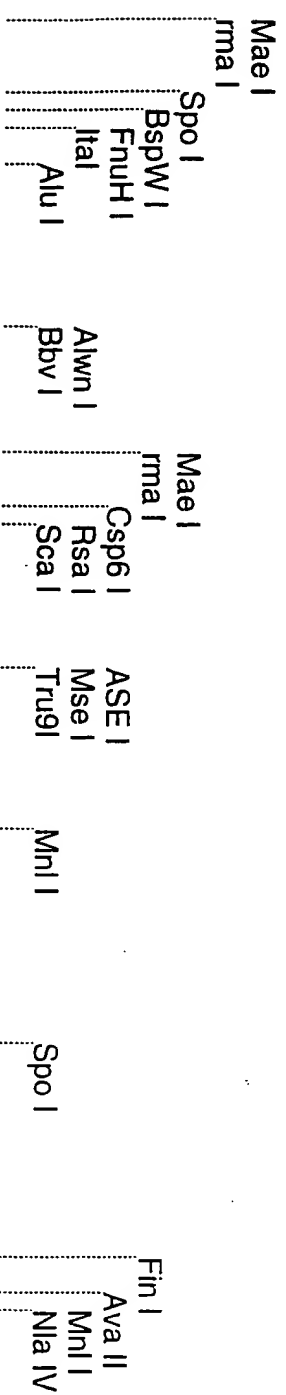
810

A G H L L Q E E T F G W D Q N N V V R S N G G D F R K T Q .  
L D T C F K K K R L D G I R I M . F D P T V G I F E K H N D  
R W T L A S R R N V W M G S E . C S S I Q R W G F S K N T M  
-----  
A P C K S . S S V N P H S . F L T T R D L P P S K R F V C H  
S S V Q K L F F R K S P I L I I Y N S G V T P P I K S F C L S  
Q V S A E L L F T Q I P D S Y H L E I W R H P N E F F V I I



900

F F F R . T A T I S I F F N Q I I I R G A K V S F M I I E S  
S S S D E P L R S V F S S I R S S S E E P R F P L . S . N R  
I L L P M N R Y D Q Y F L Q S D H H Q R S Q G F L Y D H R I  
N K K R H V A V I L I K K L . I M M L P A L T E K I I M S D  
E E E S S G S R D T N E E I L D D D S S G L N G K H D Y F R  
R R G I F R . S . Y K R . D S . . . L L W P K R . S . L I A

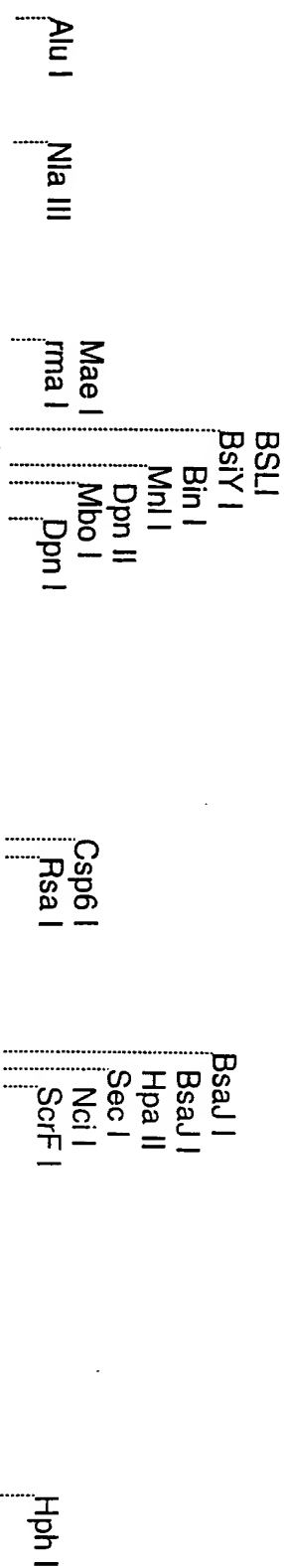


990

C T A G A C A C C T T C A G T T T C T C T T A G T A C T A T T A T T T C A A C G A G C A A C A A T C A T A C G G A C C A A T G A G A A T T T G  
C A T C T G T C A A G T C A A A G A C G A G A C A T C A T G A T T A T T A G G A A T A A G T T G C T C G T T G T T A G T A T G C C C T G T T A C C T C T T A A A C

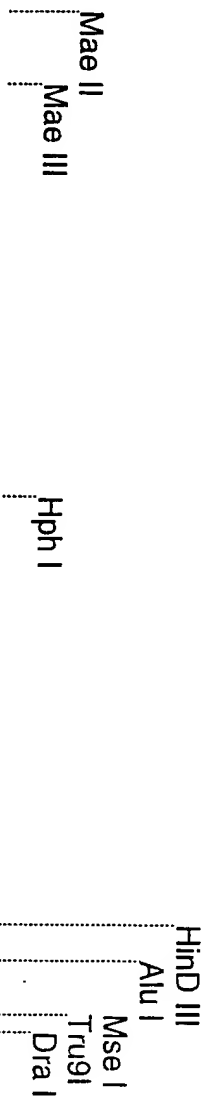
L E Q L Q F L L L V L L L I L I S T R Q Q I I R D Q W R N L  
S S F S F C F . Y Y Y . S L F Q R G N K S Y G T N G G I W  
A R A A S V S A S S T T I N P Y F N E A T N H T G P M E E F

. L L K L K Q K . Y . . . D K N . R P L L D Y P V L P P I Q  
L A A E T E A E L V V I L G . K L S A V F . V P G I S S N P



1080

G A T W K E T L E M D Q E V . R S T S F F R G N M V K E F Q  
E L H G R K P . K W I R C E G V R V F S G E I W . K S F S  
S Y M E G N P R N G S G G V K E Y E F F P G K Y G E R V S  
A V H F S V R S I S . S T H L L V L K K R P F I T F S N .  
S S C P L F G . F H I L L H S P T R T K E P S I H H F L K L  
L . M S P F G L F P D P P T F S Y S N K G P F Y P S L T E T



1170

W L Q R R H S . V I A V L I P L I C P . S F K C F I F L Y  
G G Y N V V T R R . L Q S . Y H . F V L E A L N V L S F Y I  
V A T T S S L V G D C S P N T I D L S L K L . M F Y L S I

H H S C R R . E Y T I A T R I G N I Q G Q L K L H K I K R Y  
P P . L T T V R L H N C D . Y W Q N T R S A K F T K D K . I  
T A V V D D S T P S Q L G L V M S K D K F S . I N . R E I N

BsmA I  
Mse I  
Tru9I  
Dra I  
Esp3 I  
Alw26 I  
Mse I  
Tru9I

ICATTTAAACAATAATGCTCTTTAAGAAAAACATTTTAAAGATGAAGT  
1224

ACTAAATTGGTTTACGACAGAAATTTCTTTTGGTAAATTCATCTACTTCA

. F K Q N R L F K E K T F . V D E S  
D L N K I V S L K K K H F K . M K V  
I . T K S S L . R K N I L S R . K  
N L C F R R K L S F V N . T S S L  
S K F L I T E K F F F C K L Y I F T  
I . V F D D R . L F F M K L L H F H

Titering Library

1 colony

1 RF

or

100  $\mu$ l

5 ml CB + 0.2% maltose + 10  $\mu$ M MgSO<sub>4</sub>

10 ml CB +

O/n 37°C — OD<sub>600</sub> = 1.63

37°C 2-3 hr

0.5 ml  $\rightarrow$  in 5 ml CB + 0.2% maltose + 10  $\mu$ M MgSO<sub>4</sub>

1.5 hr. OD<sub>600</sub> = 0.598

spin down 4000 rpm 4 min

10 ml MgSO<sub>4</sub>

600  $\mu$ l

14  $\mu$ l

14  $\mu$ l good

100  $\mu$ l

put phage library

150  $\mu$ l 37°C

plating

37°C 8 hr

Sequence	Primer	Primer	Time
1. CGT 226	80-26	AsT-2	100
2 CGT 226	20-31	AsT-3	100
3 SGT 282	22-	D53-2	100
4 SGT 282	22	D53-3	100
5 M3-5-1		T3 (10pm)	100
6 M3-5-1		T2	100
7 M7-1-2		T2	100
8 M7-1-2		T2	100

PCR # K1 = 60 + 1

60, 205°C

50°C

50°C

70°C

5" 25X

100°C

## Screening for SGT282 cDNA.

1° — 14 plate ( $\approx 700,000$  phages) <sup>flower library</sup> were screened with SGT282 probe (10  $\mu$ l from each PCR amplification including both 3' and 5' flanking sequences) (1  $\mu$ l library — 1000  $\mu$ l  $\rightarrow$  45  $\mu$ l/plate)

↓  
Washed 2xSSC, 1% SDS ~~for~~ 65°C 2hr.

↓  
exposed with intensifying screen, 24hr

↓  
5 positives  $\rightarrow$  500  $\mu$ l SM + chloroform 4°C  
SGT282-1, 282-2, 282-3, 282-4, 282-5

2° — positives were picked up and store in 500  $\mu$ l SM + chloroform o/n.

dilution 1  $\mu$ l — 100  $\mu$ l SM

plating: 10  $\mu$ l, 20  $\mu$ l, 50  $\mu$ l / plate respectively  
282 N- 1 2 3 (282 1-1, 1-2, 1-3)

↓  
Second Screening x 65°C hybridization, x2SSC, RT.

No. 282, 1-1-1, 1-2-1

2-1-1 -

3-1-1 -

4-1-1 -

5-1-1 - 5-1-1-2 -

↓  
Third Screening x 65°C H, x2SSC Wash RT

No. 282 3-1-1-1, ① ② ...

1/3 (50-100) were positive 4-2-4-2, ① ② — from the same plates  
5-1-1-7, ① ② -

↓  
only 3, 4, 5 were positive at the third screening  
No. 1, and 2 were missed

In Vivo excision

No. 2-1-1-1, ② 3-1-1-2 ① 3-1-1-2 ②

4-2-3-2 ① ②, 4-2-4-4 ① ② 4-2-4-4 ②



## Sequencing 282 candidate

No. of tube	Sample	Primer
<del>3</del> 1	282-3111A	T <sub>3</sub>
2	"	T <sub>7</sub>
<del>4</del> 3	282-4-1-4-2A	T <sub>3</sub> ywc 282-4 T <sub>3</sub>
4	"	T <sub>7</sub> T <sub>7</sub>
5	282-5121A	T <sub>3</sub> ywc 282-5 T <sub>3</sub>
6	"	T <sub>7</sub> ywc 282-5 T <sub>7</sub>
7	<del>28</del> M3-1A	T <sub>3</sub> ywc 3-1A T <sub>3</sub>
8	"	T <sub>7</sub> ywc 3-1A T <sub>7</sub>
9	282-5121B	T <sub>3</sub> ywc 282-5B T <sub>3</sub>
10	"	T <sub>7</sub> ywc 282-5B T <sub>7</sub>

8 µl Terminator mix  
 20 µl primer (20 µl total)  
 10 µl DNA  
 20 µl

T<sub>7</sub> promoter do not work.

New Sequencing is carried out as follows

PCR tube	Sample	Primer	mark for Label
1	282-3111A	T <sub>3</sub>	282-3A T <sub>3</sub>
2	282-3111A	T <sub>7</sub> (from Xue min)	282-3A T <sub>7</sub>
3	282-3111A	M13 Forward	282-3A MF
4	282-3111A	M13 Reverse	282-3A MR
5	282-4242A	T <sub>3</sub>	282-4A T <sub>3</sub>
6	"	T <sub>7</sub>	282-4A T <sub>7</sub>
7	"	M13 FP	282-4A MF
8	"	M13 RP	282-4A MR
9	282-4222A	T <sub>3</sub>	282-4B T <sub>3</sub>
10	"	T <sub>7</sub>	282-4B T <sub>7</sub>
11	"	M13 FP	282-4B MF
12	"	M13 RP	282-4B MR